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PREVALENCE OF *TOXOPLASMA GONDII* PARASITE INFECTION AMONG PEOPLE WHO DIED DUE TO SUDDEN DEATH IN THE CAPITAL CITY OF WARSAW AND ITS VICINITY

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ABSTRACT

BACGROUND. It has recently been shown that the behavioural effects resulting from latent *T. gondii* infection in immunocompetent people could pose as a threat to their welfare.
AIM. The aim of the study was to evaluate the prevalence of *T. gondii* infection in a group of people who died suddenly in Warsaw and its vicinity.
MATERIAL AND METHODS The studied group (n=169 people) included 42 road traffic accident victims who were driving a vehicle (bicycle (n=6), a motorbike (n=3), a motorcycle (n=13), a car (n=20)) prior to sudden death and 41 people whose death resulted from suicide. Blood samples were collected post-mortem and examined for the presence of *T. gondii,* IgG antibodies and ethyl alcohol.
RESULTS. Of the 169 people tested, *T. gondii* IgG antibodies were found in the serum of 93 (55%) of which 25 (59.5%) were drivers and 26 (63.4%) people who died as a result of suicide. With respect to the prevalence of *T. gondii* infection no statistically significant differences were found between the study (61.4%) and control group (49.4%); (p=0.09). A statistically significant result was recorded in the 38-58 age group between suicide and control groups (71.4% vs. 44.4%; $p<0.05$).
Positive test results for the presence of ethyl alcohol in the blood were reported among 49.7% of the studied population: 25.7% among drivers, 67.6% among suicides and 51.8% in the control group. To a statisctically siginificant degree, IgG *T. gondii* antibodies were found to occur more frequently in people with positive blood alcohol test results among suicides (72% vs. 50%; $p<0.05$) and among the control group (60% vs. 40%; $p<0.05$) than in their equivalents with negative test results.
CONCLUSIONS. Our work confirmed the usefulness of serologically testing samples collected post-mortem for epidemiological purposes. The small size of the study group made it impossible to evaluate the potential associations between exposure to *T. gondii* infection and the probability of sudden death. The significance of *Toxoplasma gondii* infection as a risk factor for self-destructive behaviour merits further study.

Key words*: Toxoplasma gondii, toxoplasmosis, post mortem serological testing, suicide, traffic accidents.*

INTRODUCTION

Infection with *Toxoplasma gondii* is common in the human population. The percentage of population infected is estimated to vary from 30% to 60%, depending on the region of the world (1,2). In its latent form, the infection persists until the infected person dies and is usually asymptomatic in nature. Until recently, *T. gondii* was considered to pose as a threat to the health of patients with heavy immunosuppresion and in immunonaive pregnant women, as an agent of the congenital infection. However, the results of studies carried out initially on animal models and later also on people, seem to indicate that the consequences of *T. gondii* infection can also appear in people with a properly functioning immune system. Infection among immunocompetent people are connected with the particular preference of the parasite to form cysts in the brain. It has been found that chronic *T. gondii* infection may lead to lack of concentration, prolongation of reaction time, decreased reflexes and feeling of fear (3-6). These features may lead to behaviours that threaten the health

and life of the individual. The aim of the study was to evaluate the prevalence of *Toxoplasma gondii* infection in a group of drivers examined post-mortem who died suddenly following a traffic accident as well as people who committed suicide in the Capital City of Warsaw and its vicinity.

MATERIALS AND METHODS

Blood serum samples collected post-mortem from 154 men and 15 women were examined for the presence of anti-Toxoplasma – specific IgG antibodies. The samples were collected post-mortem from people who died suddenly between May 2010 to April 2012. The study population included 42 victims from traffic accidents who were driving a vehicle including: a bicycle (n=6), a motorbike (n=3), a motorcycle (n=13), a car (n=20), at the time of the event, (hereinafter referred to as 'drivers') and 41 people whose death resulted from suicide (collectively referred to as the 'study group'). The median age for the study group was 40 years (age of death ranging from 18 – 86 years). The control group consisted of 86 people aged 20 to 89 (median = 51), who died due as a result of disease, hypothermia, ethyl alcohol intoxication or injuries sustained in a car accident (as a passenger) and being murdered (Tab I).

Table I. People examined *post mortem* for the presence of *T. gondii* IgG antibodies divided into groups: drivers, suicides and a control group with regard to age and gender.

Study group	Number of dead people examined	Age	Sex	
			F	M
1. Total number of drivers:	**42**		3	39
a. car	20	19 - 86 years (median=40)	-	20
b. motorcycle	13		1	12
c. motorbike	3		1	2
d. bicycle	6		1	5
2. Suicides	**41**	18 - 81 years (median=40)	5	36
3. Control group	**86**	20 - 89 years (median=51)	7	79

In order to test for the presence of IgG antibodies to *T. gondii* (10 ml), blood samples were collected from the cranial cavity or from the femoral vein into dry test tubes during a medico-legal examination and post-mortem examination at the Department of Forensic Medicine of Medical University of Warsaw. Serum was obtained by means of centrifugation blood clots and stored at a temperature of -20 °C until testing.

Immunoglobulin G class antibodies to *T. gondii* were detected by indirect immunofluorescence test (IF) using a second International Standard of World Health Organization (WHO IS) in accordance with the diagnostic procedure PB-03-LEP/S NIZP-PZH at the Department of Medical Parasitology of the National Institute of Public Health-National Institute of Hygiene (NIPH-NIH). The results were expressed as international units per millilitre (IU/ml). The cut off value was ≥ 1.6 IU/ml.

Ethyl alcohol blood tests collected during medico-legal and post-mortem examinations, were analysed. The tests were routinely conducted using gas chromatography as requested by the Prosecutor's Office in the Toxicology Laboratory of the Chair and Department of Forensic Medicine of Medical University of Warsaw. The tests were carried out on 155 samples obtained from: 35 drivers, 37 suicides and 83 subjects from the control group.

Statistical analysis was conducted using the Chi-squared Test (χ^2) and Mann-Whitney U Test. A p value less than 0.05 was considered statistically significant.

RESULTS

Class immunoglobulin G antibodies to *Toxoplasma gondii* were found in the blood serum of 93 (55%) of all the people studied. The percentage of positive results in the study group was 61.4% and did not differ significantly from the same figure in the control group 49.4% (p=0.09). Antibodies were detected in 25 (59.5%) drivers and 26 (63.4%) suicides.

Results from our study showed that people aged 59-76 had the highest percentage of positive results for *T. gondii* (70.3%), followed by 18-37 years (53.7%), > 77 years (50%) and 38-58 years (48.6%). No statistically significant differences were found in the prevalence of *T. gondii* infection in people in any of the specified age brackets of the study group in comparison to corresponding age brackets of the control group (p>0.05).

A statistically significant result was recorded among the group of people who committed suicide at the age of 38-58 years: the prevalence of *T. gondii* infection among these people was 71.4% while in the control group it was 44.4% (p<0,05).

Presence of ethyl alcohol in the blood was recorded in a total of 49.7% (n=77) of the study group and the control group, while in the study group alone it was 41% (n=34). In drivers, the percentage of positive results was 25.7% (n=9), in suicides – 67.6% (n=25) while in the control group – 51.8% (n=43). The mean concentration of ethyl alcohol was: in drivers 0.4 permille (‰), in suicides – 1.2‰ and in the control group – 1.2‰.

No statistically significant differences were found between the prevalence of antibodies in the group of sober drivers (54%) and in the group of inebriated driv-

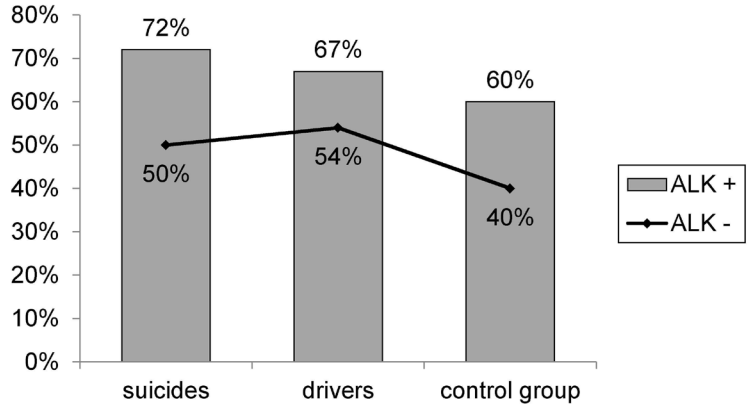

Fig 1. The prevalence of *T. gondii* IgG antibodies in the group of drivers, suicides and the control group divided into people with a positive (ALK+) and negative (ALK-) result for the present of ethyl alcohol in the blood.

ers (67%) in comparison with the control group (40% vs. 60%; $p>0.05$).

In terms of statistical significance, antibodies to were detected more frequently in people with a positive result for the presence of alcohol in their blood, than in people with a negative result who committed suicide (72% vs. 50%) and people from the control group (60% vs. 40%; $p<0.05$) (Fig 1).

DISCUSSION

Toxoplasma gondii has a predilection for brain tissue, where it creates tissue cysts. …. Animal models have shown that chronic *Toxoplasma* infections can generate changes in the behaviour of immunocompetent rodents. The infected specimens manifested a decreased feeling of fear of 'new': sounds, smells or views, increased mobility, decline in the learning ability and concentration. Also in people, behavioural disturbances were observed which may be connected to chronic toxoplasmosis. It was found that people with a confirmed presence of antibodies to *T. gondii* showed increased activity, reduced reaction time and disturbances of the personality profile (5,6).

The way in which the parasite influences behaviour is dependent on its location and the pressure generated, as studies on animal models revealed *Toxoplasma* tropism to brain structures such as nucleus amygdalae and/or nucleus accumbens which become active in conditions of fear or anxiety (7). The impact of pro-inflammatory cytokines produced in the brain in chronic infection was also studied. Other studies considered the influence of disturbances to the level of neurotransmitters, mainly dopamine, in relation to partial reduction of behavioural changes caused by *T. gondii* following the administration to animals of anti-psychotic drugs with dopaminolythic action. Recently, it was shown that *T. gondii* deregulates the level of dopamine through an extracellular secretion of tyrosine hydrolase – an enzyme taking part in the synthesis of dopamine (8).

Deregulation of the level of neurotransmitters in the brain leads to serious mood disturbances and depressive behaviours indicated as potential causes of suicides. A positive correlation was found between the presence of antibodies to *Toxoplasma* and the frequency of suicide attempts. Research carried out in Turkey revealed that the seroprevalence among people attempting suicide (41%) was significantly higher than in the control group (28%) (9).

Our study, confirmed the presence of immunoglobulin G antibodies in 26 (63.4%) of the 41 examined people who committed suicide in Warsaw and its vicinity. The percentage was higher than among people from the control group (49.4%) though the difference was not statistically significant. Men, who died as a result of suicide constituted the majority of our study group (87.8%). According to WHO statistics, approximately 80% of people who die as a result of suicide are men (10). Unfortunately, the bulk of data available concerns the prevalence of toxoplasmosis in women. Analysis of these data allowed *Ling VJ* et al. to confirm a statistically significant relation between higher prevalence of antibodies to *T. gondii* and suicidal attempts in women after menopause (11). However, it is not only toxoplasmosis but also advanced age that may predispose to more frequent suicidal attempts. *Hawton* and *van Heeringen* indicate age as a factor of significant influence on the frequency of committed suicides, particularly in men (12).

In the presented study, the percentage of men in the group of people who died as a result of suicide was 87.8% which may correspond to the percentage reported for the country in 2010 (86%) (13). The highest prevalence of *T. gondii* infections (71.4%) was found in the 38-58 age group where it was significantly higher than the prevalence in the same age bracket among people of the control group (44.4%). According to statistical data

for 2010, 49% of all suicidal attempts were reported in the 35-59 age group, with 52% of these attempts resulting in death (13).

Studies carried out in Poland by *Wojnar* et al. indicate a relationship between suicidal attempts and alcohol consumption (14). The test for the presence of ethyl alcohol in the blood was performed in 37 people who committed suicide giving a positive result in 25 of the people examined (67.6%). The prevalence of antibodies to *T. gondii* among people with a positive test for the presence of alcohol in the blood was significantly higher than in people in whom alcohol was not detected (72% vs. 50%). A similar result was obtained in the control group where the percentage of people with a positive and negative result of tests for the presence of ethyl alcohol in the blood was 60% and 40%, respectively. Abuse of alcohol often leads to impoverishment and consequently poor hygiene, both of which are listed among risk factors for toxoplasmosis. However, without more extensive research, it cannot be ruled out that contrary to this assumption it is the *Toxoplasma gondii* infection that contributes to the development of a drinking problem.

As the presence of alcohol in the blood was confirmed to contribute to an increased incidence of *T. gondii* infection, two variants to the analysis of the results of serological tests were applied: one including and the other excluding people with alcohol detected in the blood. Results were not statistically significant in either variant analysis of a higher occurrence of positive IF test results in the group of drivers who died as a result of a traffic accident in Warsaw and its vicinity in comparison with the control group. Yet, Turkish researchers confirmed a statistically higher risk of causing a traffic accident among seropositive people in the group of drivers studied (15). Also research carried out in the Czech Republic among drivers and pedestrians whose behaviour contributed to a traffic accident revealed a significantly higher incidence of chronic toxoplasmosis than in the control group (16).

Our results may be influenced by the following factors: the study population was very small, in addition certain information was lacking for the 'drivers' group such as final convictions proving the drivers were perpetrators of the car accidents in which they died. According to the information given by the Police Headquarters, drivers were found guilty in 80% of all accidents reported in 2010 (17).

CONCLUSIONS

Our study confirmed the usefulness of serologically testing samples collected post-mortem for epidemiological purposes. However the small size of the study group made it impossible to evaluate the possible associations between exposure to *T. gondii* infection and the probability of sudden death. The significance of *Toxoplasma gondii* infection as a risk factor for self-destructive behaviour merits further study.

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